Psychoneuroendocrinology 103 (2019) 14–24

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Involvement of oxytocin and GABA in consolation behavior elicited by socially defeated individuals in mandarin voles

Lai-Fu Li[a,b], Wei Yuan[a], Zhi-Xiong He[a], Li-Min Wang[a], Xiao-Yuan Jing[b], Jing Zhang[a], Yang Yang[a], Qian-Qian Guo[a], Xue-Ni Zhang[a], Wen-Qi Cai[a], Wen-Juan Hou[a], Rui Jia[a], Fa-Dao Tai[a,*]

[a] Institute of Brain and Behavioral Sciences, College of Life Sciences, Shaanxi Normal University, Xi'an, 710062, China
[b] College of Life Sciences, Nanyang Normal University, Nanyang, 473061, China

ARTICLE INFO

Keywords:
Anterior cingulate cortex
Amygdala
Consolation
Empathy
Oxytocin
GABA

ABSTRACT

Consolation, which entails comforting contact directed toward a distressed party, is a common empathetic response in humans and other species with advanced cognition. Here, using the social defeat paradigm, we provide empirical evidence that highly social and monogamous mandarin voles (*Microtus mandarinus*) increased grooming toward a socially defeated partner but not toward a partner who underwent only separation. This selective behavioral response existed in both males and females. Accompanied with these behavioral changes, c-Fos expression was elevated in many of the brain regions relevant for emotional processing, such as the anterior cingulate cortex (ACC), bed nucleus of the stria terminalis, paraventricular nucleus (PVN), basal/basolateral and central nucleus of the amygdala, and lateral habenular nucleus in both sexes; in the medial preoptic area, the increase in c-Fos expression was found only in females, whereas in the medial nucleus of the amygdala, this increase was found only in males. In particular, the GAD67/c-Fos and oxytocin (OT)/c-Fos colocalization rates were elevated in the ACC and PVN, indicating selective activation of GABA and OT neurons in these regions. The "stressed" pairs matched their anxiety-like behaviors in the open-field test, and their plasma corticosterone levels correlated well with each other, suggesting an empathy-based mechanism. This partner-directed grooming was blocked by pretreatment with an OT receptor antagonist or a $GABA_A$ receptor antagonist in the ACC but not by a V1a subtype vasopressin receptor antagonist. We conclude that consolation behavior can be elicited by the social defeat paradigm in mandarin voles, and this behavior may be involved in a coordinated network of emotion-related brain structures, which differs slightly between the sexes. We also found that the endogenous OT and the GABA systems within the ACC are essential for consolation behavior in mandarin voles.

1. Introduction

Consolation behavior has been defined as an increase in affiliative contact in response to and directed toward a distressed individual by an uninvolved bystander, which produces a calming effect (de Waal and van Roosmalen, 1979). According to Preston and de Waal's discussion of the mechanisms and levels of empathy (Preston and de Waal, 2002), consolation may represent an intermediate level of empathy (the primary level of "emotional contagion", the more complex level of "consolation", and the most elaborate level of "perspective taking and targeted helping"). It has long been assumed that this form of empathy is restricted to species possessing more complex cognitive functions such as humans, apes, elephants, dolphins, canids and corvids (for review, see de Waal and Preston, 2017). However, a recent study by Burkett et al. provided intriguing evidence that the prairie vole (*Microtus ochrogaster*), a socially monogamous North American rodent, might be capable of displaying consolation behavior (Burkett et al., 2016). In their study, the researchers demonstrated that prairie voles greatly increased grooming toward a footshock-stressed partner but not toward a partner who underwent only separation. Interestingly, these

Abbreviations: ACC, anterior cingulate cortex; ACb, nucleus accumbens; AVP, arginine vasopressin; BLA, basolateral amygdaloid nucleus; BSTd, bed nucleus of the stria terminalis dorsal part; BSTv, bed nucleus of the stria terminalis ventral part; CeA, central amygdaloid nucleus; CORT, corticosterone; DR, dorsal raphe nucleus; IL, infralimbic cortex; LHb, lateral habenular nucleus; LSv, lateral septal nucleus ventral part; MeA, medial amygdaloid nucleus; MPA, medial preoptic area; mPFC, medial prefrontal cortex; OT, oxytocin; OTR-A, OT receptor antagonist; OTR, OT receptor; PH, posterior hypothalamic area; PrL, prelimbic cortex; PVN, paraventricular nucleus; V1aR, V1a subtype of vasopressin receptor; V1R-A, vasopressin V1a receptor antagonist; VTA, ventral tegmental area

* Corresponding author.
E-mail address: taifadao@snnu.edu.cn (F.-D. Tai).

https://doi.org/10.1016/j.psyneuen.2018.12.238
Received 12 September 2018; Received in revised form 27 December 2018; Accepted 27 December 2018
0306-4530/ © 2018 Elsevier Ltd. All rights reserved.

selective responses were not observed in a closely related less-social promiscuous species of meadow vole (*Microtus pennsylvanicus*). In fact, the same response was also observed in rats, which are social but promiscuous (Knapska et al., 2010). All these results raised the question of whether consolation is seen only in social species of rodents.

The mandarin vole (*Microtus mandarinus*) is a socially monogamous rodent that is widely distributed across China. Adult mandarin voles display unique patterns of social behavior associated with a monogamous mating strategy, such as partner preference, selective aggression toward conspecific strangers, and biparental care of offspring (Tai and Wang, 2001). Therefore, this vole species provides an ideal model to investigate complex social behaviors, such as consolation. Although the most common form of stress encountered by animals in their daily lives stems from the social environment, the well-used stress paradigms in empathy-related studies have been nonsocial, including exposure to footshock, restraint or pain (Burkett et al., 2016; Donovan et al., 2018; Lu et al., 2017). Compared to these unnatural stress models, the social defeat stress paradigm has been considered to have higher ethological and pharmacological validity (Iniguez et al., 2018), but only a few studies have investigated social defeat-induced emotional contagion (Carnevali et al., 2017; Iniguez et al., 2018) and the underlying neural mechanisms are largely unknown.

Efforts have been made to examine the underlying neural mechanisms of empathy. Human brain imaging studies showed activation of several brain structures, such as the amygdala, the insular cortex and the anterior cingulate cortex (ACC), in people observing others suffering from pain or experiencing fear (Bernhardt and Singer, 2012). Animal studies have yielded similar results. In rodent models of socially transferred fear, it has been observed that c-Fos expression in the observer's amygdala and medial prefrontal cortex (mPFC, which includes the ACC, prelimbic cortex (PrL) and infralimbic cortex (IL) subregions) generally mirrors the changes observed in those who experienced the footshock (Mikosz et al., 2015); the ACC limited deletion of the Cav1.2 type 1 Ca2+ channels, contributing to synaptic transmission and neural excitability, impaired vicarious fear learning in mice (Jeon et al., 2010); in prairie voles, socially interacting with a stressed partner resulted in elevated c-Fos expression in the ACC, but not in the PrL or nucleus accumbens (ACb) (Burkett et al., 2016). The patterns of brain activation seem to be species specific and to vary according to the subjects' sex, social experiences and the test paradigms (Christov-Moore et al., 2014; Jones and Monfils, 2016; Meyza et al., 2017). Therefore, a description of the brain activation patterns that occur in response to the consolation behavior induced by the social defeat paradigm would be interesting and valuable.

One molecular substrate of empathy is oxytocin (OT). In addition to its typical reproductive roles, OT is also closely associated with a broad range of social behaviors in rodents, such as social approaches (Teng et al., 2013), attachment (He et al., 2017) and buffering (Smith and Wang, 2014). Burkett's study indicated that OT participates in footshock-induced consolation behaviors through the OT receptor (OTR) within the ACC (Burkett et al., 2016). However, receptors for the structurally similar neuropeptide vasopressin (AVP) are also found in the mPFC of a variety of microtine species (Smeltzer et al., 2006). Both OT and AVP modulate a variety of complex social behaviors (Neumann and Landgraf, 2012), and some studies showed that some behavioral effects of OT involve the V1a subtype of the vasopressin receptor (V1aR) (Bowen and McGregor, 2014; Ramos et al., 2013). Therefore, it remains to be determined whether V1aR in the ACC is also involved in consolation behavior.

Furthermore, the mechanism by which OT mediates consolation within the mPFC remains unclear. Several lines of evidence suggest that OT may interact with GABA, the main inhibitory neurotransmitter in the brain, to exert its biological effects (Dong et al., 2017; Sabihi et al., 2017; Smith et al., 2016). Recent studies have also shown that the OTR is located on GABAergic interneurons in the cortex (Marlin et al., 2015; Nakajima et al., 2014), where OT has been found to increase GABA levels (Qi et al., 2012). When combined with the observation that some empathy-impairing diseases are associated with decreases in GABA function (Paine et al., 2017) and are alleviated by OT treatment (Yamasue et al., 2018), it is reasonable to hypothesize that OT exerts its effects on consolation via GABAergic pathways in the ACC.

The present study was conducted to address these questions. In Experiment 1, we first examined whether consolation behaviors could be elicited in mandarin voles by exposure to a defeated partner and then investigated the associated brain activation patterns. In Experiment 2, we examined whether the behavioral changes in bystander observers are empathy based by assaying for some of the purported characteristics, such as emotional contagion, state matching, familiarity bias and self-other differentiation (de Waal and Preston, 2017). In Experiment 3, we examined whether microinjection of OTR, V1aR and $GABA_A$ receptor antagonists in the ACC could impair the consolation behaviors elicited by the social defeat paradigm.

2. Material and methods

2.1. Ethics statement and animals

The mandarin voles used in this experiment were laboratory bred from generations of ancestors derived from a wild population from Lingbao (Henan, China). The voles were weaned on postnatal day 21 and socially housed with two to three animals in each polycarbonate cages (44 × 22 × 16 cm). The subjects were maintained on a 12 light/12 dark photoperiod with unlimited access to food (carrots and rabbit chow). The voles were used for experiments after reaching adulthood (70–90 days of age). All breeding, housing, and experimental procedures were approved by the Animal Care and Use Committee of Shaanxi Normal University in accordance with the Guide for the Care and Use of Laboratory Animals of China. All efforts were made to minimize suffering and the number of animals used.

2.2. Behavioral scoring

Digital videos from all experiments were viewed and quantified by raters who were blinded to the experimental groups and treatments using *J* Watcher software (http://www.jwatcher.ucla.edu/). Videos were coded for allogrooming or other behaviors, such as self-grooming, huddling, chasing, sniffing and digging, depending on the experiment. Allogrooming was defined as head contact with the body or head of another individual, accompanied by a rhythmic head movement. All behavioral studies were conducted under dim light during the dark phase of the light-dark cycle (approximately 20:00—22:00 h).

2.3. Experiment 1

This experiment was designed to investigate whether reunion with a defeated partner would alter the behavioral and neural activity of the subject. This study was conducted in both males and females.

2.3.1. Consolation test

Before testing began, adult male and female voles were cohoused for four days to allow for the formation of a pair bond (Yu et al., 2012). In each mated pair, one vole was designated as "observer", and the other vole was designated as "demonstrator". The observers were marked by cutting a bundle of hair on the back. The home cages were moved into the testing room. The subjects were then left undisturbed for 20 min of environmental adaptation. The demonstrators were then removed from their cages, transferred into an acoustically isolated room, placed in an empty cage with clean bedding, and left undisturbed for 15 min. After this time period, the demonstrators were reunited with their respective partners for 10 min in the test room. The pairs were then returned to their original rearing room thereafter. This procedure was repeated for three consecutive days for habituation.

On the 4th day, the subjects underwent an identical procedure, except for the following changes. The demonstrators were randomly assigned to one of two experimental conditions: (1) a social defeat stress (n = 10 for each sex) or (2) a control (n = 10 for each sex). For subjects in the experimental stressor groups, the demonstrator (designated as stressed "demonstrator") was placed for 15 min in the cage of an aggressive same-sex vole (resident), whose partner had previously been removed. The demonstrator voles were then attacked and defeated by the resident, i.e., when the intruder vole assumed a supine or upright posture that was held for at least 5 s (Wang et al., 2018). In contrast, the controls (designated as control "demonstrators") were placed for 15 min in an empty cage with soiled bedding. The soiled bedding was used to rule out any confounding effects related to olfactory stimuli originating from other voles. The demonstrators were then reunited with their respective partners (designated as stressed or control "observers", respectively) for 10 min in the test room. The behaviors of the observers were digitally recorded during this process. Then, the demonstrators were removed, and the observers were left alone in their home cages for another 80 min, at which time they were euthanized and perfused as described below for immunofluorescence experiments. The study protocol and timeline of the procedures were adapted from Burkett's study (Burkett et al., 2016) and are depicted in Supplemental Fig. 1.

2.3.2. Immunofluorescence

To minimize the number of animals used, only the first six observers in each group were euthanized for immunofluorescence studies. In detail, the subjects were anesthetized with 2% sodium pentobarbital and then transcardially perfused with 0.1 M PBS (pH 7.4), followed by 4% paraformaldehyde. The brains were rapidly removed and immersed in the same fixative for 3 days at 4 °C and then dehydrated in 15% and then 30% freshly prepared sucrose solutions. Transverse slices of the brain were cut at 40 μm using a cryostat (CM-1850, Leica, Germany). We used c-Fos, an immediate early gene, as a cellular marker of neural activity and GAD67 (the enzyme that synthesizes GABA from glutamate) as a cellular marker of GABA neurons. Fos-positive neurons were examined on serial sections from rostral (A/P: approximately + 2.5 mm from bregma) to caudal (A/P: approximately –4.5 mm from bregma) with an interval of 120 μm. The regions of interest were emotion-related structures, including but not limited to the mPFC, ACb, septal nucleus, bed nucleus of the stria terminalis (BST), medial preoptic area (MPA), PVN, amygdala, habenula, hippocampus, ventral tegmental area (VTA), and dorsal raphe nucleus (DR). To investigate whether OT and GABA neurons were activated during this process, double staining of GAD67/c-Fos and OT/c-Fos was conducted in the mPFC and PVN, respectively. The detailed immunofluorescence procedure and quantifications can be found in the Appendix.

2.4. Experiment 2

This experiment was designed to investigate whether the behavioral changes in observers were empathy-based. As allogrooming behavior did not differ between the sexes (Results, Section 3.1), only males were designated as observers (n = 9 in each group). The timeline of the study is depicted in Supplemental Fig. 1.

2.4.1. Consolation test

The consolation test protocol was similar to that used in Experiment 1, except that the reunion time was shortened to 5 min to guarantee high levels of corticosterone (CORT), which is in accordance with Burkett's study (Burkett et al., 2016). We used "active behavior" to indicate emotional arousal in this experiment, which included allogrooming, self-grooming, chasing, sniffing, walking and running, and digging.

2.4.2. Anxiety-like behavior assay

We used both an open-field test and a social approach and avoidance test to evaluate anxiety-like behaviors. While the open-field test emphasizes exploration and relies on the rodents' innate fear of open spaces (nonsocial), the social approach and avoidance test places emphasizes anxiety in the social context. After the consolation test, the 5-min open-field test was conducted, followed by the two 5-min phases of the social approach and avoidance test. The sequence of the tests was chosen to eliminate the potential effect of stimulus voles in the social approach and avoidance test on a subsequent open-field test (if the tests followed the reverse sequence). The detailed study procedures are presented in the Appendix.

2.4.3. Plasma collection and CORT assay

After the social approach and avoidance test, the animal was taken into another room and anesthetized with isoflurane, and retro-orbital blood was collected in a heparin-coated tube within 2 min by a trained experimenter. The tube was then centrifuged at 3000 rpm at 4 °C for 15 min to collect the plasma, which was stored at − 40 °C until analysis for the determination of plasma CORT levels. A commercially available ELISA kit (Enzo, USA) was used to quantify the levels of CORT in the plasma. Only samples with a coefficient of variation less than 10% were included in the analysis.

2.5. Experiment 3

This experiment was designed to investigate whether OTR, V1aR and $GABA_A$ receptor activation in the ACC was required for the observed consolation behavior. As in Experiment 2, only males were designated as observers. We used three cohorts of animals in this experiment. There was a cohort for each drug and each cohort had a separate saline control group. The detailed procedure is shown below, and the timeline is summarized in Supplemental Fig. 1B.

2.5.1. Stereotaxic cannulation

In general, the voles were anesthetized with an isoflurane/air mixture and mounted on a stereotaxic apparatus. Bilateral cannula guide tubes (0.41 mm o.d. × 0.25 mm i.d.) with 1.0 mm separation were implanted aimed toward the ACC (A/P: 1.6 mm, M/L: ± 0.5 mm, and D/V: 1.8 mm; from the bregma). After surgery, the animals were housed with free access to food and water and were allowed to recover for four days before being paired with an age-matched female demonstrator.

2.5.2. Microinjection and behavior assay

Observers were habituated to the consolation test protocol used in Experiment 1 for three days, as described above. On the last day of testing, the observers were anesthetized with an isoflurane/air mixture and given a bilateral injection of vehicle (saline, 0.2 μL/side); vehicle containing an OT receptor antagonist (OTR-A; [d(CH2)5, Tyr(Me)2, Thr4, Tyr-NH2 9]-OVT; Bachem, Switzerland) (0.5, 5 and 50 ng/200 μL per side); a vasopressin V1a receptor antagonist (V1R-A ; [phenylacetyl1, O-Me-D-Tyr2, Arg6,8, Lys9]-vasopressin amide; Sigma, USA) (0.5, 5 and 50 ng/200 μL per side); or bicuculline (a specific $GABA_A$ receptor antagonist, Sigma, USA) (0.5, 2.5 and 25 ng/200 μL per side). The observers were then allowed 5 min to recover from anesthesia before being moved to the testing room and then administered a consolation test as described above. The dose and timing of drug administration were chosen based on previous studies with OTR-A (Burkett et al., 2016; Sabihi et al., 2017), bicuculline (Dong et al., 2017; Palotai et al., 2014), and AVPR-1a (Donaldson et al., 2010; Manning et al., 2012).

Then, an open-field test was conducted to determine whether the locomotor activity was affected by the drugs. Finally, cannula placement was confirmed through histological localization of the guide cannula on slide-mounted brain sections. Only subjects with the correct cannula placements and normal movement capacities were included in

the final data analysis ($n = 5$ in each group).

2.6. Statistical analyses

In general, all data were assessed for normality using a one-sample Kolmogorov-Smirnov test, and Levene's test was used to confirm homogeneity of variance. Unless explicitly stated, ANOVAs was the default analysis method. Independent-samples *t*-tests (normal data, '*t*' values are provided) or Mann–Whitney *U* tests (nonnormal data, '*Z*' values are provided) were used to evaluate the intended comparisons. Bonferroni corrections were conducted for multiple comparisons when appropriate. The relationships of plasma CORT concentrations between observers and demonstrators were determined by Pearson's correlation analysis, and the two correlations were compared using Fisher's transformation test. Most of the statistical procedures were performed using SPSS 20.0 (SPSS Inc., Chicago, USA). The significance level was set at $P < 0.05$.

3. Results

3.1. Behavioral performance

The behavioral performance of male and female observers in the consolation test is depicted in Fig. 1. Two-way ANOVA revealed a significant main effect of condition (stressed or control) on both the frequency of and the time spent on allogrooming ($F_{(1,36)} = 54.7$ and 49.1, respectively; all $P < 0.01$) and chasing ($F_{(1,36)} = 11.7$ and 5.5; $P < 0.01$, $P < 0.05$, respectively). Stressed observers of both sexes showed a higher frequency of and more time spent on allogrooming than controls ($t_{(18)} = 7.8$ and 4.2 for males, $t_{(18)} = 3.2$, $Z = -2.8$ for females, frequencies reported first for both sexes, all $P < 0.01$). However, only stressed female observers showed a higher frequency of ($Z = -2.9$, $P < 0.01$; Fig. 1C) and more time spent on ($Z = -2.5$, $P < 0.05$; Fig. 1D) chasing after the defeated partner. Stressed male observers also showed such tendencies in chasing, but the difference did not reach the significant level (frequency: 4.7 ± 1.4 *vs*. 2.6 ± 1.0, time percent: 2.2% ± 0.7% *vs*. 1.4% ± 0.4%, stressed *vs*. control; Fig. 1A & B). No other differences were observed in the frequency of or time spent on grooming, huddling, sniffing and digging between the stressed and control observers in both sexes.

3.2. c-Fos expression patterns

We found that c-Fos was substantially expressed in the ACC region of the mPFC, ACb, lateral septal nucleus ventral part (LSv), BST, MPA, PVN, amygdala, posterior hypothalamic area (PH), lateral habenular nucleus (LHb), VTA and DR. The main findings are summarized in Table 1.

Table 1
c-Fos positive neurons in selected brain structures of male and female voles upon exposure to a control or a social defeated partner.

Brain region	Male		Female	
	Control	Stressed	Control	Stressed
mPFC				
ACC	16.2 ± 2.4	61.7 ± 6.8**	11.8 ± 2.6	59.8 ± 5.0**
PrL	9.2 ± 1.7	8.1 ± 1.1	8.0 ± 1.0	9.5 ± 1.3
IL	8.6 ± 1.0	8.3 ± 1.2	6.5 ± 0.8	7.7 ± 1.3
Acb				
Core	37.8 ± 3.4	48.3 ± 8.4	41.0 ± 4.1	45.9 ± 4.5
Shell	29.5 ± 8.7	36.0 ± 8.4	23.3 ± 3.6	27.5 ± 4.1
LSv	66.8 ± 6.4	60.0 ± 8.3	82.2 ± 15.4	91.0 ± 12.6
BST				
BSTd	18.8 ± 1.9	60.5 ± 17.0*	19.2 ± 4.4	69.4 ± 8.8**
BSTv	15.3 ± 3.1	67.8 ± 25.0*	22.8 ± 1.9	95.8 ± 7.5**
MPA#	20.5 ± 5.4	38.3 ± 6.6	27.6 ± 2.6	105.8 ± 5.9**
PVN	51.8 ± 4.6	93.3 ± 12.7*	37.8 ± 4.8	74.0 ± 8.5**
Amygdala				
CeA#	17.3 ± 1.9	39.0 ± 2.7**	14.5 ± 1.8	22.3 ± 2.6*
MeA	34.5 ± 6.8	75.2 ± 10.0*	63.7 ± 9.3	79.8 ± 10.9
BLA	18.8 ± 1.5	37.8 ± 5.0*	35.3 ± 3.0	52.3 ± 11.0*
PH	67.5 ± 14.1	71.0 ± 5.1	78.5 ± 17.4	97.0 ± 6.2
LHb	10.5 ± 1.2	114.7 ± 12.7**	27.5 ± 5.9	68.7 ± 10.1**
VTA	44.6 ± 9.4	56.0 ± 6.9	38.0 ± 9.4	37.2 ± 8.1
DR	30.5 ± 5.5	35.0 ± 7.6	27.5 ± 6.7	21.3 ± 5.7

Data are presented as mean ± SE. *$P < 0.05$, **$P < 0.01$ for within-sex comparisons; #$P < 0.05$ for between sex comparisons; $N = 4$–6 in each group. ACb: nucleus accumbens; ACC: anterior cingulate cortex; BLA: basolateral amygdaloid nucleus; BSTd: bed nucleus of the stria terminalis dorsal part; BSTv: bed nucleus of the stria terminalis ventral part; CeA: central amygdaloid nucleus; DR: dorsal raphe nucleus; IL: infralimbic cortex; LHb: lateral habenular nucleus; LSv: lateral septal nucleus ventral part; MeA: medial amygdaloid nucleus; MPA: medial preoptic area; mPFC: medial prefrontal cortex; PH: posterior hypothalamic area; PrL: prelimbic cortex; PVN: paraventricular nucleus; VTA: ventral tegmental area.

In the ACC, two-way ANOVA revealed a significant main effect of condition ($F_{(1,20)} = 103.8$, $P < 0.01$). Follow-up analyses revealed that c-Fos expression was significantly elevated in the stressed observers in both sexes ($Z = -2.8$ and –2.9, respectively; all $P < 0.01$). There was little c-Fos expression in the PrL and IL regions of the mPFC in both sexes.

Similar to the ACC, c-Fos expression was also elevated in both the dorsal and ventral parts of the BST (BSTd and BSTv) in the stressed

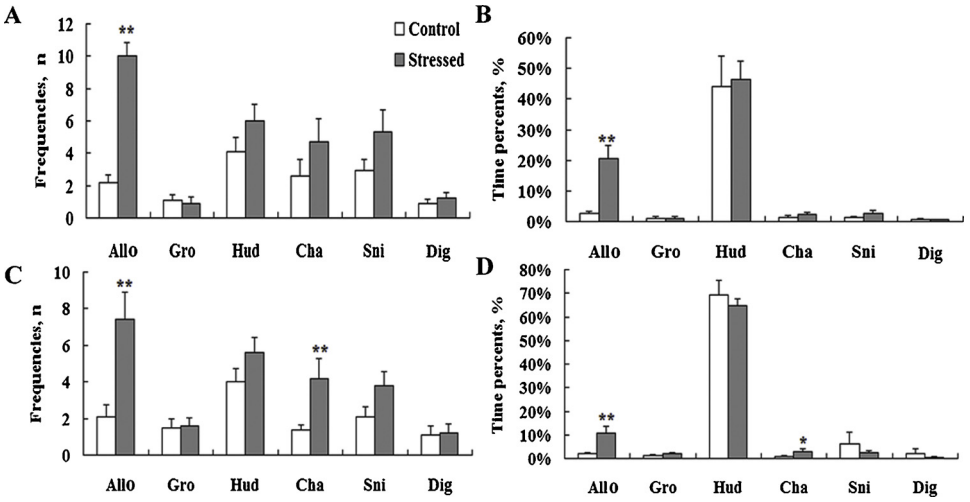

Fig. 1. The frequencies and percent time of the observers' behaviors during the 10-min consolation test in male (A and B) and female (C and D) voles. Data are presented as the mean ± SE, $n = 10$ in each group, **$P < 0.01$ and *$P < 0.05$ compared to controls. CTR: control; STR: stressed; Allo: allogrooming; Gro: grooming; Hud: huddling; Cha: chasing; Sni: Sniffing; Dig: digging.

observers of both sexes ($Z = -2.3$ and -2.4, respectively, for males, all $P < 0.05$; $Z = -2.6$ and -2.5, respectively, for females, all $P < 0.01$).

The amygdala was divided into the BLA, central amygdaloid nucleus (CeA) and medial amygdaloid nucleus (MeA) sections. In the BLA, there were significant main effects of condition ($F_{(1,12)} = 25.1$, $P < 0.01$) and sex ($F_{(1,12)} = 7.7$, $P < 0.05$). Follow-up analyses revealed that c-Fos expression was significantly higher in the stressed observers in both sexes ($Z = -2.3$ and -2.2 for males and females, respectively; all $P < 0.05$). The results in the CeA were similar to those in the BLA except that the overall c-Fos expression was higher in male observers for the between-sex comparison (Bonferroni test, $P < 0.05$). However, the situation was somewhat different in the MeA, which showed a main effect only for condition ($F_{(1,20)} = 9.2$, $P < 0.01$), and the c-Fos expression was elevated only in the stressed male observers ($Z = -2.4$, $P < 0.05$).

In the MPA, ANOVA yielded significant effects of condition ($F_{(1,14)} = 83.9$, $P < 0.01$), sex ($F_{(1,14)} = 50.8$, $P < 0.01$), and sex × condition interactions ($F_{(1,14)} = 33.3$, $P < 0.01$). For between-sex comparisons, the results revealed that c-Fos expression was higher in female observers than that in male observers (Bonferroni test, $P < 0.05$). For within-sex comparisons, the results revealed that c-fos expression was elevated only in stressed female observers ($Z = -2.6$, $P < 0.01$).

In the PVN, the data showed a significant main effect of condition ($F_{(1,20)} = 21.5$; $P < 0.01$), and c-Fos expression was significantly higher in the stressed observers in both sexes ($Z = -2.2$, $P < 0.05$ for males; $Z = -2.8$, $P < 0.01$ for females).

In the LHb, there was a significant main effect of condition ($F_{(1, 20)} = 114$, $P < 0.01$) and the sex × condition interaction ($F_{(1, 20)} = 23.7$, $P < 0.01$). c-Fos expression was significantly higher in the stressed observers in both sexes ($Z = -2.9$ and -2.7, males reported first; all $P < 0.01$).

Although a quantity of c-Fos was expressed in the ACb (both the core and shell sections), PH, VTA and DR, no significant effects of condition, sex or sex × condition were observed.

3.3. Double staining

The double staining results are presented in Fig. 2. The detailed merged images can be found in Supplemental Figs. 2 (PVN) and 3 (ACC).

In the ACC, two-way ANOVA revealed a significant main effect of condition on GAD67/c-Fos colocalization ($F_{(1,20)} = 33.1$, $P < 0.01$). Follow-up analyses revealed that the colocalization rates were significantly higher in stressed observers in both sexes ($Z = -2.9$, $P < 0.01$ for males, $Z = -2.4$, $P < 0.05$ for females, Fig. 2F). There was hardly any GAD67/c-Fos colocalization in the PrL and IL regions of the mPFC (data not shown).

In the PVN, the results resembled those in the ACC, which showed a significant effect of condition ($F_{(1,20)} = 28.9$, $P < 0.01$). Similarly, OT/c-Fos colocalization rates were significantly higher in stressed observers in both sexes ($Z = -2.4$, $P < 0.05$ for males, $Z = -2.8$, $P < 0.01$ for females, Fig. 2E).

3.4. Experiment 2

3.4.1. Consolation test results

The 5-min consolation test results are presented in Fig. 3. Two-way ANOVA yielded significant effects of group (observer or demonstrator), condition (stressed or control), and the group × condition interaction on allogrooming time ($F_{(1,32)} = 51.8$, 59.9 and 54.7, respectively; all $P < 0.01$). After exposure to a defeated partner, the total activity time of the stressed observers was significantly elevated ($t_{(16)} = 4.8$, $P < 0.01$) (Fig. 3A). Again, the observers spent more time allogrooming the defeated demonstrators (Fig. 3B, $t_{(16)} = 7.3$, $P < 0.01$ compared to the control). The total activity and allogrooming time were not different between the defeated and control demonstrators ($t_{(16)} = 0.915$ and 0.271, respectively, all $P > 0.05$).

3.4.2. Anxiety behavior test

The open-field test and social approach and avoidance test results are presented in Fig. 4. In the open-field test, two-way ANOVA yielded significant effects of condition on both the time spent in the central zones ($F_{(1,32)} = 24.1$, $P < 0.01$) and the total distance moved ($F_{(1,32)} = 7.3$, $P < 0.05$). In particular, both the stressed observers and demonstrators spent much less time in the central zones than their respective controls ($t_{(16)} = -2.3$, $P < 0.05$ and $t_{(16)} = -5.124.1$, $P < 0.01$; Fig. 4A); only stressed demonstrators traveled a shorter distance than control demonstrators ($t_{(16)} = -2.4$, $P < 0.05$; Fig. 4B).

In the social approach and avoidance test, when the stimulus vole was absent (object stimulus), three-way ANOVA results revealed no group, condition, zone or interaction effects on the time spent in the interaction zone; however, when the stimulus vole was present (social stimulus), significant effects of group ($F_{(1,64)} = 7.8$, $P < 0.01$), condition ($F_{(1,64)} = 6.9$, $P < 0.05$), zone ($F_{(1,64)} = 9.0$, $P < 0.01$), condition × zone ($F_{(1,64)} = 4.0$, $P < 0.05$), group × zone ($F_{(1,64)} = 4.0$, $P < 0.05$) and group × condition × zone ($F_{(1,64)} = 7.7$, $P < 0.05$) were found. Specifically, the stressed demonstrators spent much less time in the interaction zone ($t_{(16)} = -5.6$, $P < 0.01$) than control demonstrators; no differences were found between the stressed and control observers (Fig. 4D). All the observers and control demonstrators spent more time in the interaction zone when the stimulus was present (all $P < 0.05$ vs. object stimulus), and this effect was not observed in the defeated demonstrators ($t_{(16)} = 1.6$, $P > 0.05$).

3.4.3. CORT levels in the ELISA test

The ELISA test results are depicted in Fig. 5. There were significant effects of group ($F_{(1,20)} = 8.1$, $P < 0.01$) and condition ($F_{(1,20)} = 5.1$, $P < 0.05$). Overall, observers (male) have higher CORT levels than demonstrators (female), regardless of treatment ($t_{(22)} = 0.017$, $P < 0.05$), which may indicate a sex difference. Specifically, the plasma CORT levels in the defeated demonstrators were significantly higher than those in the control demonstrators ($Z = -2.0$, $P < 0.05$). Although the mean value of plasma CORT levels in the stressed observers was higher than that in the control observers (0.95 ± 0.25 vs. 0.57 ± 0.19 μg/mL), this difference did not reach significance ($Z = -1.1$, $P > 0.05$, Fig. 5A). Regression correlation analyses showed that the plasma CORT levels in the stressed observers strongly correlated with those in the defeated demonstrators ($R^2 = 0.805$, $P < 0.05$, Fig. 5B), but this correlation was not observed in the control observer/demonstrator pairs ($R^2 = 0.024$, $P > 0.05$). The Fisher's transformation test revealed a significant difference between these two correlations ($Z = 2.2$, $P < 0.05$).

3.5. Experiment 3

The pharmacological results are summarized in Fig. 6. One-way ANOVA revealed that microinjection of OTR-A into the ACC affected the observers' allogrooming time ($F_{(3,16)} = 6.5$, $P < 0.01$). Follow-up analysis revealed that both medium (5 ng) and high (50 ng) doses significantly reduced the allogrooming time (Tukey's test, all $P < 0.01$ vs. vehicle control), whereas low-dose (0.5 ng) administration had no effect ($P > 0.05$ vs. vehicle control; Fig. 6D). Bicuculline treatments also affected the allogrooming time ($F_{(3,16)} = 3.3$, $P < 0.05$), but only at a high doses (25 ng/side; Tukey's test, $P < 0.01$ vs. vehicle control; Fig. 6E). All three selected doses of V1R-A had no effect on the observers' allogrooming time (Fig. 6C). Further open-field test results indicated that the injections had no effect on the movement of the subjects (data not shown).

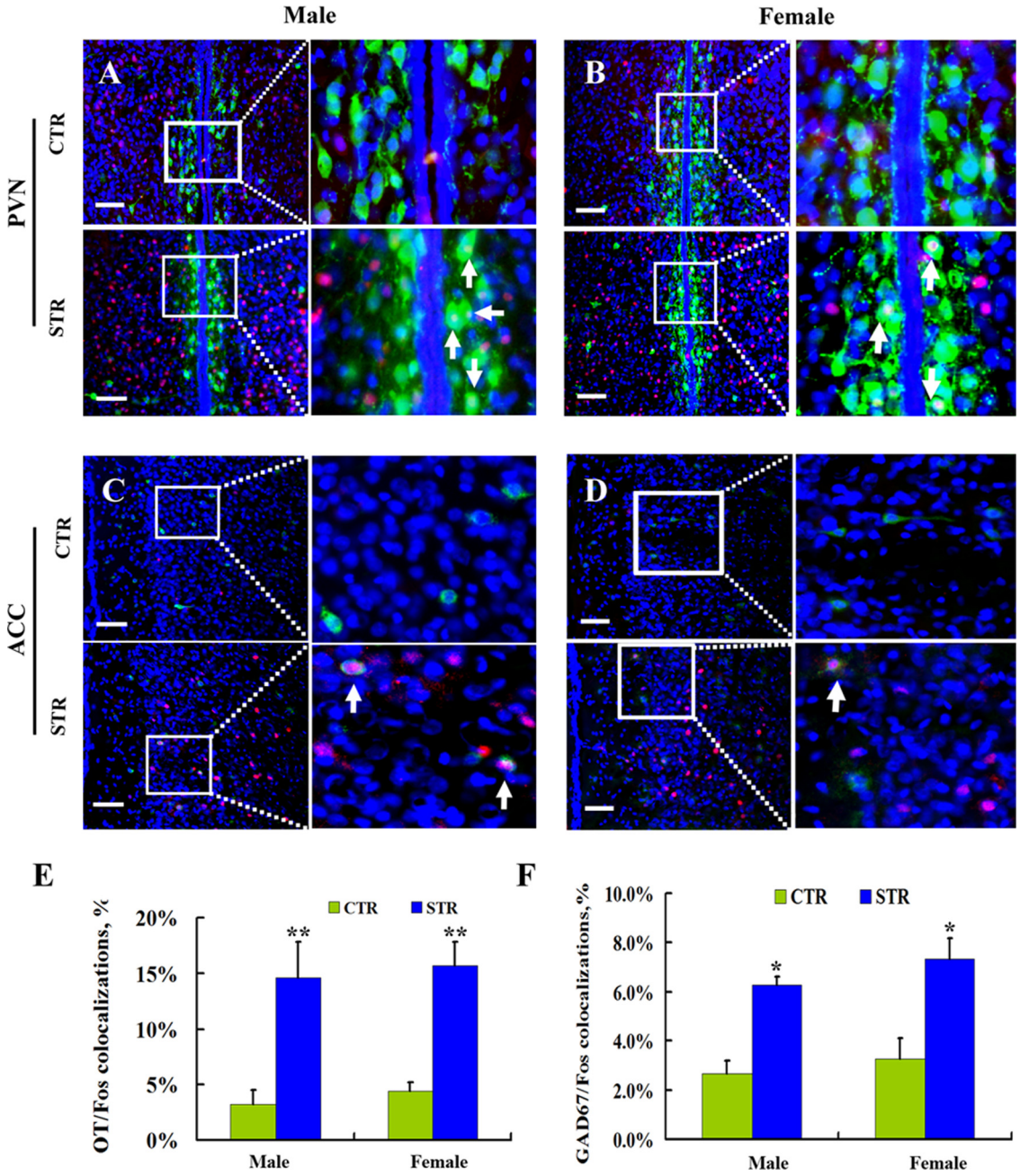

Fig. 2. Observers exposed to a defeated partner show elevated OT/c-Fos and GAD67/c-Fos colocalization rates in the PVN and ACC, respectively, in both sexes. (A and B): Representative images of OT (green)/c-Fos (red dots) colocalization in the PVN of control (top row) and "stressed" observers (bottom row); (C and D): Representative images of GAD67 (green)/c-Fos (red dots) colocalizations in the ACC of control (top row) and "stressed" observers (bottom row); (E and F): Quantification of OT/c-Fos and GAD67/c-Fos colocalization rates in the PVN and ACC, respectively. The arrows indicate double-labeled cells (light yellow); bar = 200 μm. Data are presented as the mean ± SE, $n = 6$ in each group, $^{**}P < 0.01$ and $^{*}P < 0.05$ compared to the respective controls. CTR: control; STR: stressed; ACC: anterior cingulate cortex; PVN: paraventricular nucleus (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

4. Discussion

In this study, we explored consolation behavior and its underlying neuroendocrine mechanisms in a social defeat transferred fear model in mandarin voles. Our main and novel findings are that after interaction with a defeated cagemate: (1) the naive observer greatly increased grooming toward the defeated partner; (2) c-Fos expression levels were elevated in many of the brain regions relevant to emotional processing, with sex differences in some brain structures; (3) OT and GABA neurons were activated in the PVN and ACC; (4) the behavior changes in observers seem to be empathy based; and (5) this partner-directed response was blocked by pretreatment with an OT receptor antagonist or a $GABA_A$ receptor antagonist in the ACC.

4.1. Consolation behavior could be elicited by a defeated partner

In Experiment 1, we found that the observers of both sexes spent significantly more time and showed a higher frequency of grooming their distressed partners, but this behavior was not observed toward partners who underwent separation alone. Our findings were highly consistent with Burkett's study in prairie voles (Burkett et al., 2016) and with Knapska's study in a socially but nonmonogamous species of rat

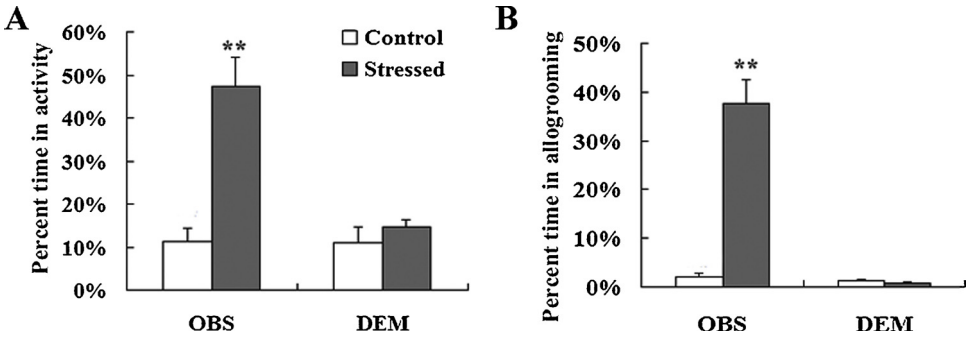

Fig. 3. Stressed male observers show increased activity (A) and allogrooming (B) in the 5-min consolation test. Data are presented as the mean ± SE, $n = 9$ in each group, $^{**}P < 0.01$ compared to the respective controls. OBS: observers; DEM: demonstrators.

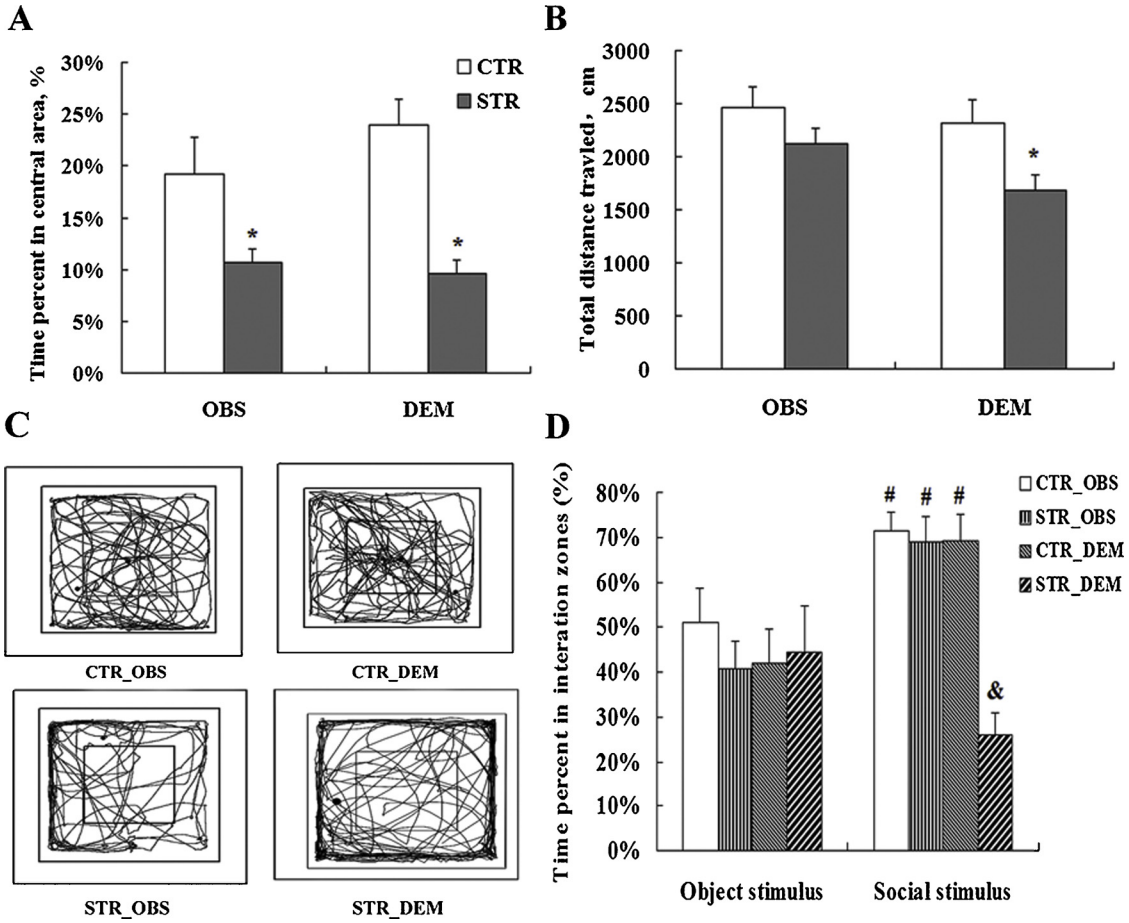

Fig. 4. The behavior of observers and demonstrators in the open-field test and the social approach and avoidance test. Time spent in the central area (A) and total distance moved (B) in the open-field test and a representative track (C). (D): Time spent in the interaction zones with (social) and without (object) stimulus voles in the social approach and avoidance test. $N = 9$ in each group, $^{*}P < 0.05$ compared to the respective controls in the open-field test; $^{\#}P < 0.05$ for comparisons between the social stimulus and the object stimulus within the respective groups in the social approach and avoidance test, $^{\&}P < 0.05$ for comparisons between control and defeated demonstrators. CTR: control; STR: stressed; OBS: observers; DEM: demonstrators.

(Knapska et al., 2010), which used electric footshock as a stressor. Intriguingly, no such increase in allogrooming was observed in a non-social nonmonogamous species of meadow vole (Burkett et al., 2016). These results support the hypothesis that consolation may exist only in social species of rodents, irrespective of their mating patterns (Perez-Manrique and Gomila, 2018). A strength of this study was that we used the social-defeat paradigm, which has more ethological and pharmacological significance than the above artificial stress paradigms (Iniguez et al., 2018).

The stressed female observers also increased chasing (following) their defeated partner, which were not observed in their male counterparts. We think this discrepancy between the sexes may be simply due to type II error or insufficient statistical power resulting from limited subjects, as stressed male observers also showed such tendency (Fig. 1A & B).

4.2. Brain activation patterns

Along with the behavioral changes in the stressed observers, some brain structures were activated, which included but were not limited to the ACC, amygdala, PVN, BST, MPA and LHb.

ACC. A growing body of evidence indicates that the ACC

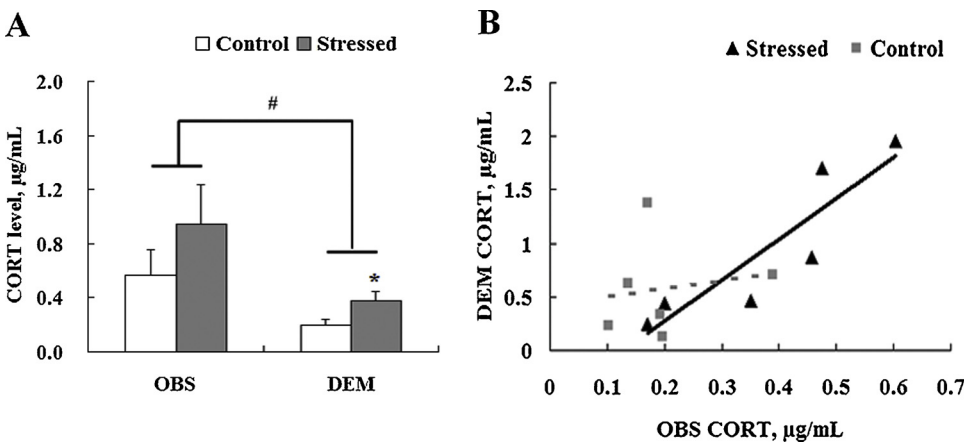

Fig. 5. Plasma CORT levels are elevated in defeated demonstrators (A) and correlate well with those in their stressed partners (B). $N = 6$ in each group, $^{*}P < 0.05$ for comparisons between control and defeated demonstrators; $^{\#}P < 0.05$ for comparisons between observers (male) and demonstrators (female). CORT: corticosterone; OBS: observers; DEM: demonstrators.

participates in socially transferred pain, fear or anxiety in rodents (Jeon et al., 2010; Pisansky et al., 2017). In line with this evidence, c-Fos expression in the ACC was significantly elevated in the stressed observers. The ACC seems to be involved in the consolation behavior, regardless of the type of stressor suffered by demonstrators. However, the c-Fos expression patterns in the PrL and IL seem to be species and sex specific in response to empathy-like behaviors. In a socially transferred fear model in mice, c-Fos expression was heightened in the PrL and IL in stressed male "observers" (Meyza et al., 2015); similar effects were present in male rats, but no such changes were observed in females (Mikosz et al., 2015). In this study, we found little c-Fos expression in the PrL and IL in both sexes, which is consistent with Burkett's study (Burkett et al., 2016). Although species differences may account for this discrepancy, other factors, such as the protocols and apparatuses used in the studies, may also play a role in this discrepancy.

Amygdala. In our study, increased c-Fos protein levels were found in the BLA and CeA of stressed observers of both sexes; however, in the MeA, the elevated expression was found only in males. MeA is a central hub for rodent social odor communication and shows sexual dimorphism (Janak and Tye, 2015). The differential c-Fos expression observed here may indicate the sex-specific processing of social cues, as reported in mice (Bergan et al., 2014). In rats, Mikosz et al. found that c-Fos expression was heightened in the BLA and MeA in males but not in females (Mikosz et al., 2015). However, in mice, this increase was observed only in the BLA (Meyza et al., 2015). Structurally, the BLA receives substantial input from the mPFC and projects to both the CeA and MeA; and all of these subregions play important roles in fear expression, learning and extinction (Janak and Tye, 2015). A recent study by Allsop et al. found that ACC—BLA circuits are essential for observational learning and social interaction in mice (Allsop et al., 2018). Therefore, BLA activation may be a process that enables socially transmitted fear/anxiety. Overall, the sex-, species- and region-specificity of c-Fos expression patterns in the amygdala may reflect different strategies of fear transmission that are employed by animals that

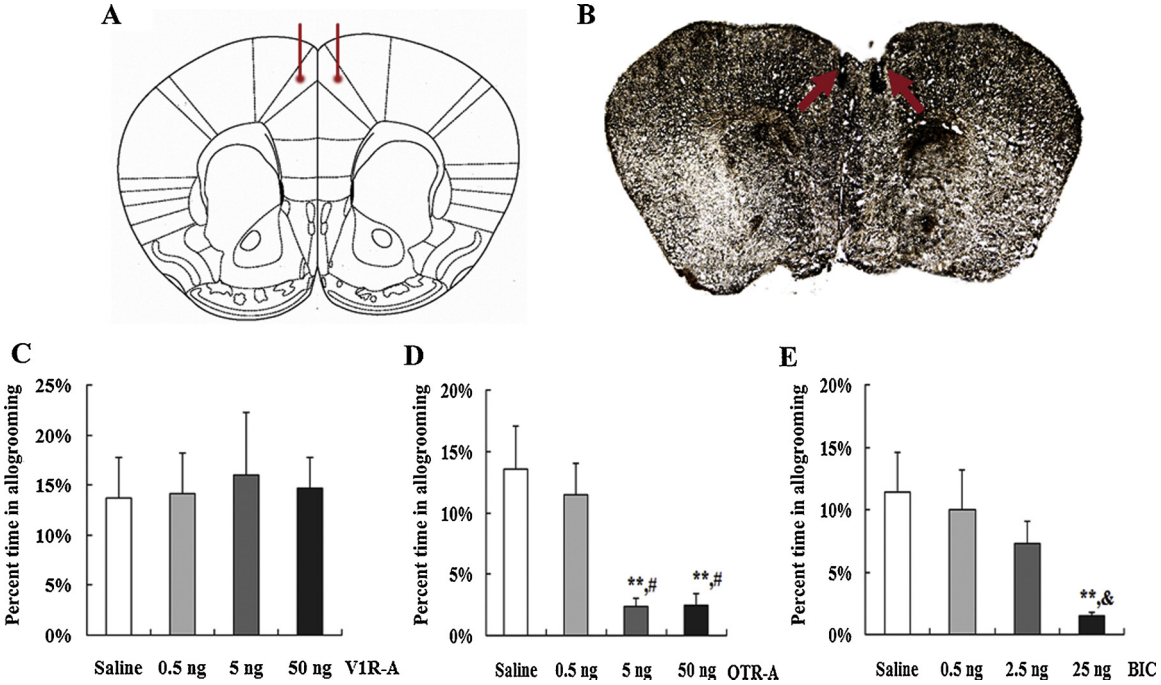

Fig. 6. The effects of microinjection of V1R-A, OTR-A and bicuculline in the ACC on the time spent in allogrooming in male observers. (A): Schematic representation of ACC infusion sites; (B): A representative photomicrograph of the injection site (× 20); (C–E): Dose-dependent effects of V1R-A, OTR-A and bicuculline. Data are presented as the mean ± SE, $n = 5$ in each group; the red arrows indicate cannula locations. $^{**}P < 0.01$ compared to the saline control; $^{\#}P < 0.05$ compared to the 0.5 ng of OTR-A; $^{\&}P < 0.05$ compared to the 0.5 ng of BIC. ACC: anterior cingulate cortex; BIC: bicuculline; OTR-A: OT receptor antagonist; V1R-A: vasopressin V1a receptor antagonist (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

evolved under particular social and evolutionary conditions.

PVN. It is well known that the PVN is critically involved in mediating stress responses. Therefore, increased c-Fos expression may be a physiological indicator of anxiety in stressed observers. Another interesting finding was that the c-Fos/OT colocalization rate was elevated in the PVN (Fig. 2). This result indicated that OT neurons are activated when this prosocial behavior occurs. Our results are in accordance with those of a recent study in mice, which showed that stimulation of PVN OT neurons with DREADDs can elicit observational fear (Pisansky et al., 2017). Considering that OT-positive fibers originating from the PVN project to the forebrain structures (Knobloch et al., 2012), it is tempting to propose that the socially transferred fear/anxiety activates the OT system in the PVN, causing OT release in the ACC, which ultimately leads to the consolation behavior. Endogenous OT may also be released into other brain structures, such as the CeA and BST, which elicit region-specific behaviors (Knobloch et al., 2012).

MPA. In the MPA, the c-Fos expression patterns in the stressed observers were sex specific, with an increase in females but no difference in males. The MPA receives inputs from the MeA and BST and sends outputs to the VTA, which play important roles in maternal behavior, pair bonding and affect modulation (Ch'ng et al., 2018). In fact, the current view is that the evolutionary roots of empathy are based on maternal care of their young (de Waal and Preston, 2017; Meyza et al., 2017). Therefore, it is not surprising that the MPA was activated in the stressed female observers. Although c-Fos expression showed a slight increase in the stressed male observers, this difference did not reach statistical significance. We also found that the overall c-Fos expression was lower in the stressed male observers than in their female counterparts. The sex difference here may arise from sexual dimorphism in the underlying neural circuits, which has been implicated in the MPA of mice (Scott et al., 2015).

BST and LHb. The BST receives substantial input from the mPFC, BLA, CeA, VTA and DR and is considered to be a hub center of emotion and reward in rodents (Lebow and Chen, 2016). The other interesting finding is that c-Fos expression was also elevated in the LHb. The LHb has been described as a relay interface between the basal ganglia and the limbic system and plays multiple roles in maternal behavior, motivation, anxiety, and depression (Yang et al., 2018). Recent studies have demonstrated that this nucleus plays an important role in negative reward learning, which is activated by unpleasant events (Matsumoto and Hikosaka, 2009). The increased c-Fos expression here may therefore indicate the experience of negative emotions in response to their defeated partners.

Generally, our studies expanded upon the results of previous studies by showing that in addition to the traditional ACC/amygdala brain structures, additional limbic brain structures, such as the PVN, BST, MPA and LHb, were also activated, accompanied by consolation behavior, in response to a defeated partner in mandarin voles. These findings provide new avenues for examining the neural substrates for empathy-like behaviors in the future.

4.3. Consolation behaviors in mandarin voles are empathy based

As consolation is a higher level of empathy, we next investigated whether the behavioral changes in the stressed observers had an empathy-based mechanism, which would include emotional contagion, state matching, familiarity bias, and self-other differentiation (de Waal and Preston, 2017). In accordance with this possibility, observers interacting with a defeated demonstrator showed increased activity compared to the controls (Fig. 3A). This emotional arousal or vigilance state induced by the stressed partner may be an example of emotional contagion. The other evidence that the observer was not neutral to the stressed state of the demonstrator was that the stressed observers showed signs of anxiety in the open-field test, which matched the behavior of their defeated partners (Fig. 4A). Furthermore, the plasma CORT levels in the stressed observers strongly correlated with those of the defeated demonstrators (Fig. 5B), representing another example of physiological state matching. However, the plasma CORT levels in the stressed observers were not different from those of control observers, and the stressed observers also showed no signs of social anxiety in the social approach and avoidance test (Fig. 4D). This result makes sense because in sympathetic concern the behavioral displays and anxiety indicators of the bystander are expected to be less overt than those of the distressed party (i.e., a moderate other-oriented emotional reaction) (de Waal and Preston, 2017). The lack of social anxiety in the social approach and avoidance test could also because social stimuli are very salient to highly social animals; therefore, this test may not be sensitive to mild stressors in mandarin voles.

Although both stressed observers and demonstrators showed some signs of anxiety (in the case of the open-field test for the observers) during the reunion, observers increased allogrooming toward demonstrators, but the demonstrators themselves did not have altered levels of allogrooming (Fig. 3B). This differential response dependent on the source of the individual's stress (vicarious or personal) is an example of self-other differentiation (Burkett et al., 2016). Empathy-related responses and behaviors are biased toward familiar individuals in many species (de Waal and Preston, 2017), but this bias cannot be tested in mandarin voles as the initial and primary responses are 'attack' in unfamiliar encounters.

It should be noted that a calming effect of consolation was not observed in this study. Even though they were intimately groomed by their partner, the defeated demonstrators showed behavioral (Fig. 4) and physiological (Fig. 5A) signs of anxiety. This finding probably occurred because social defeat is a serious physical and psychological stressor for individuals (Wood and Bhatnagar, 2015) or because the consolation time was too short (5 min) to detect a social buffering effect.

4.4. OTR and GABAA receptors in the ACC are necessary for consolation

It has been demonstrated that OTR activation in the ACC is necessary for the expression of empathy-like behavior (Burkett et al., 2016; Pisansky et al., 2017). In line with this evidence, our results indicated that OTR antagonist administration in the ACC abolished the consolation response in observers. V1aR may not be involved in this process, as treatment with different doses of a V1aR antagonist had no effect on allogrooming behavior. Another interesting finding of this study was that the endogenous GABA system in the ACC may also play an essential role in consolation behavior. In Experiment 1, we found that the increased allogrooming behavior was accompanied by increased activation of GABA neurons in the ACC. In Experiment 3, we found that the consolation response in the stressed observers could be blocked by treatment with a $GABA_A$ receptor antagonist. Considering that previous studies have found OTR on only GABAergic interneurons in the cortex (Nakajima et al., 2014), it is reasonable to speculate that the OT-GABA pathway plays an essential role in consolation behavior. In fact, this pathway has also been previously shown to mediate the anxiolytic action of OT within the amygdala (Knobloch et al., 2012), PVN (Smith et al., 2016) and PrL (Sabihi et al., 2017), suggesting that a similar mechanism is present across these brain regions, even for different behaviors. How does this pathway work? Recent studies have shown that OT increases the release of GABA (Qi et al., 2012), which induces phase-locked neuronal firing and coordinates action potentials in groups of neurons (Ryan et al., 2012), and this process may ultimately enable consolation behavior. However, the exact mechanisms and the relevant neuronal circuit should be verified in future studies.

4.5. General discussion and conclusions

Overall, the results of this study demonstrated that consolation behavior could be elicited by a defeated partner in mandarin voles and that the OT-GABA pathway within the ACC may be necessary for the

expression of this consolation behavior. The limitations of this study are worth noting to avoid overstating the results. First, only male observers were used to examine whether the observers' responses were empathy based in Experiment 2 and in the pharmacological studies of the neural substrates in Experiment 3. Therefore, it is not clear whether similar mechanisms also function in females. Second, we examined consolation only with mates, and whether this behavior exists in other types of familiar relationships, such as siblings and unrelated long-term cagemates, still needs to be verified. Third, given the small sample size presented the Fos data in Table 1, this portion of the work maybe just exploratory, and more studies should be conducted to verify the results. Finally, although we tried to control estrus states of female voles by exposing to a male partner for the same amount of time, the effects of the estrus cycle on consolation behavior still need further examination. Overall, the availability of a social defeat model to study consolation behavior in mandarin voles provides an excellent opportunity to further study the neural circuitry and neurochemical mechanisms that underlie this empathy-like behavior, for which deficits are present in many psychiatric diseases, such as autism, schizophrenia, and psychopathy.

Conflict of interest

None.

Author statement

Prof. Tai FD designed the study; Li LF conducted the majorities of experiments and wrote the original draft; Yuan W participated in the ELISA experiment; He ZX and Jia R discussed the results and provided constructive comments; Wang LM participated in the immunofluorescence experiment; Jing XY, Yang Y, Zhang J, Zhang XN, Guo QQ, Cai WQ and Hou WJ participated in the behaviors' study and helped to collect and analyze the data. All authors contributed to and have approved the final manuscript.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (31372213 and 31670421). We thank Yuan Wang and Yu-Ying Yang for assistance in conducting experiments and caring for voles and AJE® for help in polishing the language in this manuscript.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2018.12.238.

References

Allsop, S.A., Wichmann, R., Mills, F., Burgos-Robles, A., Chang, C.J., Felix-Ortiz, A.C., Vienne, A., Beyeler, A., Izadmehr, E.M., Glober, G., Cum, M.I., Stergiadou, J., Anandalingam, K.K., Farris, K., Namburi, P., Leppla, C.A., Weddington, J.C., Nieh, E.H., Smith, A.C., Ba, D., Brown, E.N., Tye, K.M., 2018. Corticoamygdala transfer of socially derived information gates observational learning. Cell 173, 1329–1342 e1318.

Bergan, J.F., Ben-Shaul, Y., Dulac, C., 2014. Sex-specific processing of social cues in the medial amygdala. eLife 3, e02743.

Bernhardt, B.C., Singer, T., 2012. The neural basis of empathy. Annu. Rev. Neurosci. 35, 1–23.

Bowen, M.T., McGregor, I.S., 2014. Oxytocin and vasopressin modulate the social response to threat: a preclinical study. Int. J. Neuropsychopharmacol. 17, 1621–1633.

Burkett, J.P., Andari, E., Johnson, Z.V., Curry, D.C., de Waal, F.B., Young, L.J., 2016. Oxytocin-dependent consolation behavior in rodents. Science 351, 375–378.

Carnevali, L., Montano, N., Statello, R., Coude, G., Vacondio, F., Rivara, S., Ferrari, P.F., Sgoifo, A., 2017. Social stress contagion in rats: Behavioural, autonomic and neuroendocrine correlates. Psychoneuroendocrinology 82, 155–163.

Ch'ng, S., Fu, J., Brown, R.M., McDougall, S.J., Lawrence, A.J., 2018. The intersection of stress and reward: BNST modulation of aversive and appetitive states. Prog. Neuropsychopharmacol. Biol. Psychiatry 87, 108–125.

Christov-Moore, L., Simpson, E.A., Coude, G., Grigaityte, K., Iacoboni, M., Ferrari, P.F., 2014. Empathy: gender effects in brain and behavior. Neurosci. Biobehav. Rev. 46 (Pt 4), 604–627.

De Waal, F.B.M., Preston, S.D., 2017. Mammalian empathy: behavioural manifestations and neural basis. Nat. Rev. Neurosci. 18, 498–509.

De Waal, F.B.M., van Roosmalen, A., 1979. Reconciliation and consolation among chimpanzees. Behav. Ecol. Sociobiol. 5, 55–66.

Donaldson, Z.R., Spiegel, L., Young, L.J., 2010. Central vasopressin V1a receptor activation is independently necessary for both partner preference formation and expression in socially monogamous male prairie voles. Behav. Neurosci. 124, 159–163.

Dong, N., Du, P., Hao, X., He, Z., Hou, W., Wang, L., Yuan, W., Yang, J., Jia, R., Tai, F., 2017. Involvement of GABAA receptors in the regulation of social preference and emotional behaviors by oxytocin in the central amygdala of female mandarin voles. Neuropeptides 66, 8–17.

Donovan, M., Liu, Y., Wang, Z., 2018. Anxiety-like behavior and neuropeptide receptor expression in male and female prairie voles: the effects of stress and social buffering. Behav. Brain Res. 342, 70–78.

He, Z., Hou, W., Hao, X., Dong, N., Du, P., Yuan, W., Yang, J., Jia, R., Tai, F., 2017. Oxytocin receptor antagonist treatments alter levels of attachment to mothers and central dopamine activity in pre-weaning mandarin vole pups. Psychoneuroendocrinology 84, 124–134.

Iniguez, S.D., Flores-Ramirez, F.J., Riggs, L.M., Alipio, J.B., Garcia-Carachure, I., Hernandez, M.A., Sanchez, D.O., Lobo, M.K., Serrano, P.A., Braren, S.H., Castillo, S.A., 2018. Vicarious social defeat stress induces depression-related outcomes in female mice. Biol. Psychiatry 83, 9–17.

Janak, P.H., Tye, K.M., 2015. From circuits to behaviour in the amygdala. Nature 517, 284–292.

Jeon, D., Kim, S., Chetana, M., Jo, D., Ruley, H.E., Lin, S.Y., Rabah, D., Kinet, J.P., Shin, H.S., 2010. Observational fear learning involves affective pain system and Cav1.2 Ca2+ channels in ACC. Nat. Neurosci. 13, 482–488.

Jones, C.E., Monfils, M.H., 2016. Dominance status predicts social fear transmission in laboratory rats. Anim. Cogn. 19, 1051–1069.

Knapska, E., Mikosz, M., Werka, T., Maren, S., 2010. Social modulation of learning in rats. Learn. Mem. 17, 35–42.

Knobloch, H.S., Charlet, A., Hoffmann, L.C., Eliava, M., Khrulev, S., Cetin, A.H., Osten, P., Schwarz, M.K., Seeburg, P.H., Stoop, R., Grinevich, V., 2012. Evoked axonal oxytocin release in the central amygdala attenuates fear response. Neuron 73, 553–566.

Lebow, M.A., Chen, A., 2016. Overshadowed by the amygdala: the bed nucleus of the stria terminalis emerges as key to psychiatric disorders. Mol. Psychiatry 21, 450–463.

Lu, Y.F., Yang, Y., Li, C.L., Wang, Y., Li, Z., Chen, J., 2017. The locus coeruleus-norepinephrine system mediates empathy for pain through selective up-regulation of P2X3 receptor in dorsal root ganglia in rats. Front. Neural Circuits 11, 66.

Manning, M., Misicka, A., Olma, A., Bankowski, K., Stoev, S., Chini, B., Durroux, T., Mouillac, B., Corbani, M., Guillon, G., 2012. Oxytocin and vasopressin agonists and antagonists as research tools and potential therapeutics. J. Neuroendocrinol. 24, 609–628.

Marlin, B.J., Mitre, M., D'Amour, J.A., Chao, M.V., Froemke, R.C., 2015. Oxytocin enables maternal behaviour by balancing cortical inhibition. Nature 520, 499–504.

Matsumoto, M., Hikosaka, O., 2009. Representation of negative motivational value in the primate lateral habenula. Nat. Neurosci. 12, 77–84.

Meyza, K., Nikolaev, T., Kondrakiewicz, K., Blanchard, D.C., Blanchard, R.J., Knapska, E., 2015. Neuronal correlates of asocial behavior in a BTBR T (+) Itpr3(tf)/J mouse model of autism. Front. Behav. Neurosci. 9, 199.

Meyza, K.Z., Bartal, I.B., Monfils, M.H., Panksepp, J.B., Knapska, E., 2017. The roots of empathy: through the lens of rodent models. Neurosci. Biobehav. Rev. 76, 216–234.

Mikosz, M., Nowak, A., Werka, T., Knapska, E., 2015. Sex differences in social modulation of learning in rats. Sci. Rep. 5, 18114.

Nakajima, M., Gorlich, A., Heintz, N., 2014. Oxytocin modulates female sociosexual behavior through a specific class of prefrontal cortical interneurons. Cell 159, 295–305.

Neumann, I.D., Landgraf, R., 2012. Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. Trends Neurosci. 35, 649–659.

Paine, T.A., Swedlow, N., Swetschinski, L., 2017. Decreasing GABA function within the medial prefrontal cortex or basolateral amygdala decreases sociability. Behav. Brain Res. 317, 542–552.

Palotai, M., Telegdy, G., Jaszberenyi, M., 2014. Orexin A-induced anxiety-like behavior is mediated through GABA-ergic, alpha- and beta-adrenergic neurotransmissions in mice. Peptides 57, 129–134.

Perez-Manrique, A., Gomila, A., 2018. The comparative study of empathy: sympathetic concern and empathic perspective-taking in non-human animals. Biol. Rev. Camb. Philos. Soc. 93, 248–269.

Pisansky, M.T., Hanson, L.R., Gottesman, I.I., Gewirtz, J.C., 2017. Oxytocin enhances observational fear in mice. Nat. Commun. 8, 2102.

Preston, S.D., de Waal, F.B., 2002. Empathy: its ultimate and proximate bases. Behav. Brain Sci. 25, 1–20 discussion 20-71.

Qi, J., Han, W.Y., Yang, J.Y., Wang, L.H., Dong, Y.X., Wang, F., Song, M., Wu, C.F., 2012. Oxytocin regulates changes of extracellular glutamate and GABA levels induced by methamphetamine in the mouse brain. Addict. Biol. 17, 758–769.

Ramos, L., Hicks, C., Kevin, R., Caminer, A., Narlawar, R., Kassiou, M., McGregor, I.S., 2013. Acute prosocial effects of oxytocin and vasopressin when given alone or in combination with 3,4-methylenedioxymethamphetamine in rats: involvement of the V1A receptor. Neuropsychopharmacology 38, 2249–2259.

Ryan, S.J., Ehrlich, D.E., Jasnow, A.M., Daftary, S., Madsen, T.E., Rainnie, D.G., 2012. Spike-timing precision and neuronal synchrony are enhanced by an interaction between synaptic inhibition and membrane oscillations in the amygdala. PLoS One 7, e35320.

Sabihi, S., Dong, S.M., Maurer, S.D., Post, C., Leuner, B., 2017. Oxytocin in the medial

prefrontal cortex attenuates anxiety: anatomical and receptor specificity and mechanism of action. Neuropharmacology 125, 1–12.
Scott, N., Prigge, M., Yizhar, O., Kimchi, T., 2015. A sexually dimorphic hypothalamic circuit controls maternal care and oxytocin secretion. Nature 525, 519–522.
Smeltzer, M.D., Curtis, J.T., Aragona, B.J., Wang, Z., 2006. Dopamine, oxytocin, and vasopressin receptor binding in the medial prefrontal cortex of monogamous and promiscuous voles. Neurosci. Lett. 394, 146–151.
Smith, A.S., Wang, Z., 2014. Hypothalamic oxytocin mediates social buffering of the stress response. Biol. Psychiatry 76, 281–288.
Smith, A.S., Tabbaa, M., Lei, K., Eastham, P., Butler, M.J., Linton, L., Altshuler, R., Liu, Y., Wang, Z., 2016. Local oxytocin tempers anxiety by activating GABAA receptors in the hypothalamic paraventricular nucleus. Psychoneuroendocrinology 63, 50–58.
Tai, F.D., Wang, T.Z., 2001. Social organization of mandarin voles in burrow system. Acta Theriol. Sin. 21, 50–56.
Teng, B.L., Nonneman, R.J., Agster, K.L., Nikolova, V.D., Davis, T.T., Riddick, N.V., Baker, L.K., Pedersen, C.A., Jarstfer, M.B., Moy, S.S., 2013. Prosocial effects of oxytocin in two mouse models of autism spectrum disorders. Neuropharmacology 72, 187–196.
Wang, L., Hou, W., He, Z., Yuan, W., Yang, J., Yang, Y., Jia, R., Zhu, Z., Zhou, Y., Tai, F., 2018. Effects of chronic social defeat on social behaviors in adult female mandarin voles (*Microtus mandarinus*): Involvement of the oxytocin system in the nucleus accumbens. Prog. Neuropsychopharmacol. Biol. Psychiatry 82, 278–288.
Wood, S.K., Bhatnagar, S., 2015. Resilience to the effects of social stress: evidence from clinical and preclinical studies on the role of coping strategies. Neurobiol. Stress 1, 164–173.
Yamasue, H., Okada, T., Munesue, T., Kuroda, M., Fujioka, T., Uno, Y., Matsumoto, K., Kuwabara, H., Mori, D., Okamoto, Y., Yoshimura, Y., Kawakubo, Y., Arioka, Y., Kojima, M., Yuhi, T., Owada, K., Yassin, W., Kushima, I., Benner, S., Ogawa, N., Eriguchi, Y., Kawano, N., Uemura, Y., Yamamoto, M., Kano, Y., Kasai, K., Higashida, H., Ozaki, N., Kosaka, H., 2018. Effect of intranasal oxytocin on the core social symptoms of autism spectrum disorder: a randomized clinical trial. Mol. Psychiatry 2018 (June). https://doi.org/10.1038/s41380-018-0097-2. [Epub ahead of print].
Yang, Y., Wang, H., Hu, J., Hu, H., 2018. Lateral habenula in the pathophysiology of depression. Curr. Opin. Neurobiol. 48, 90–96.
Yu, P., An, S., Tai, F., Zhang, X., He, F., Wang, J., An, X., Wu, R., 2012. The effects of neonatal paternal deprivation on pair bonding, NAcc dopamine receptor mRNA expression and serum corticosterone in mandarin voles. Horm. Behav. 61, 669–677.